Supporting Information

The Presence of Fast-Exchanging Proton Species in Aqueous Solutions of paraCEST Agents Can Impact Rate Constants Measured for Slower Exchanging Species When Fitting CEST Spectra to the Bloch Equations

Osasere M. Evbuomwan,[*,†] *Joohwan Lee,*[†] *Mark Woods,*[‡,#] *and A. Dean Sherry*[*†,§]

[†] Department of Chemistry, University of Texas at Dallas, 800 W. Campbell Road, Richardson, TX 75080

[‡] Department of Chemistry, Portland State University, 1719 SW 10th Ave, Portland, OR 97201

[#] Advanced Imaging Research Center, Oregon Health and Science University, 3181 S. W. Sam Jackson Park Road, Portland, OR 97239

[§] Advanced Imaging Research Center, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235

EXPERIMENTAL

Synthesis and NMR studies

Ligands 1 and 2 and their respective europium complexes were synthesized using published procedures.[1,2] High resolution ^{1}H-NMR spectra were recorded in D_2O on a Bruker AVANCE III 400 NMR spectrometer operating at 400.13 MHz. CEST spectra were generated by applying a frequency-selective saturation pulse in 1 ppm increments over a 200 ppm frequency range, and plotting the residual bulk water signal intensity ratio (M_s / M_o) as a function of saturation frequency. NMR samples containing different proportions of Eu-1 and Eu-2 were prepared from 20 mM stock solutions and CEST resulting from water exchange was measured at 25^{o}C and pH 7. A saturation time of 6 s was used while the saturation power was varied from 400 – 1000 Hz. The CEST properties of serial dilutions of Eu-1 were also studied as a control under the same experimental conditions.

Calculation of the water exchange rate constants (k_{ex})

The water exchange rate constants (k_{ex}) were calculated by fitting the acquired CEST spectra to a concentration dependent 3-pool model (representing the bulk water pool, the Eu^{3+}-bound water pool, and the amide protons) based on the NMR Bloch equations modified for exchange. A fit of the data to a 3-pool model consisting of the bulk water pool, the Eu^{3+}-bound water pool of the SAP isomer, and the Eu^{3+}-bound water pool of the TSAP isomer was also examined. For comparison, k_{ex} values were also determined using a concentration independent method referred to as the omega method [3]. The bulk water signal intensity at the CEST exchange site was expressed as a fraction of the residual initial magnetization (M_s / M_o – M_s) and this relationship was plotted as a function of the reciprocal square of the saturation power (1 / ω_1^2). The data was

fit to a linear equation and the k_{ex} value was estimated by calculating the inverse of the square root of the x-intercept.

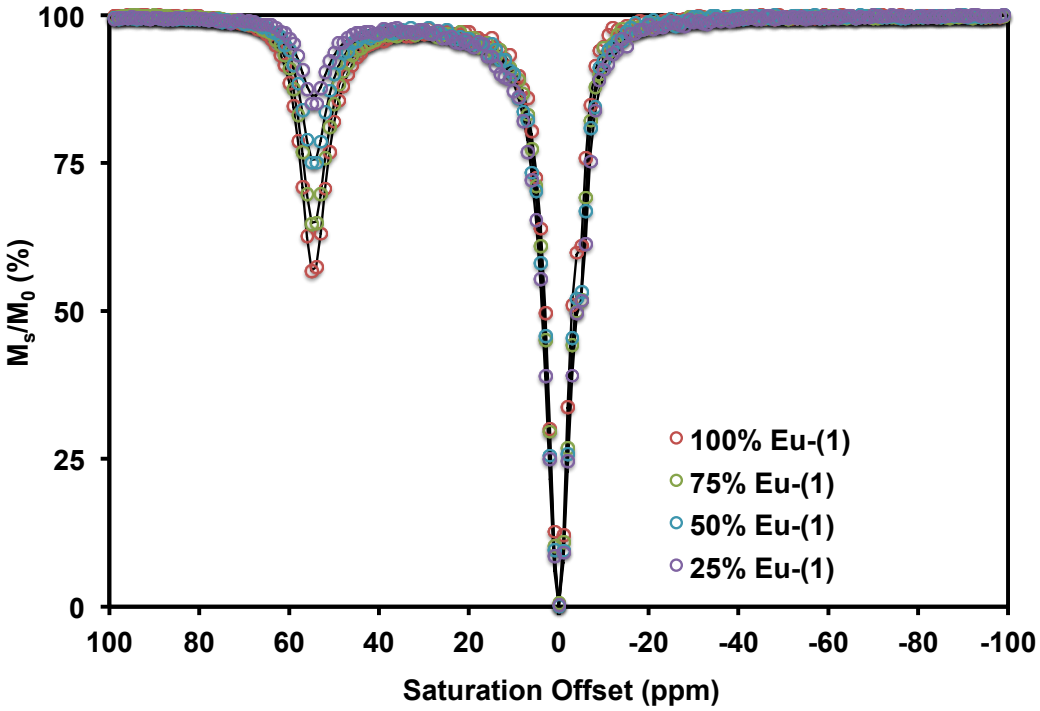

Figure S1. CEST spectra of aqueous solutions containing different proportions of Eu-(1) and Eu-(2) with [Eu^{3+}] = 20 mM. Spectra were recorded at 25 °C, pH 7, B_1 = 9.4 μT, saturation time = 6 s.

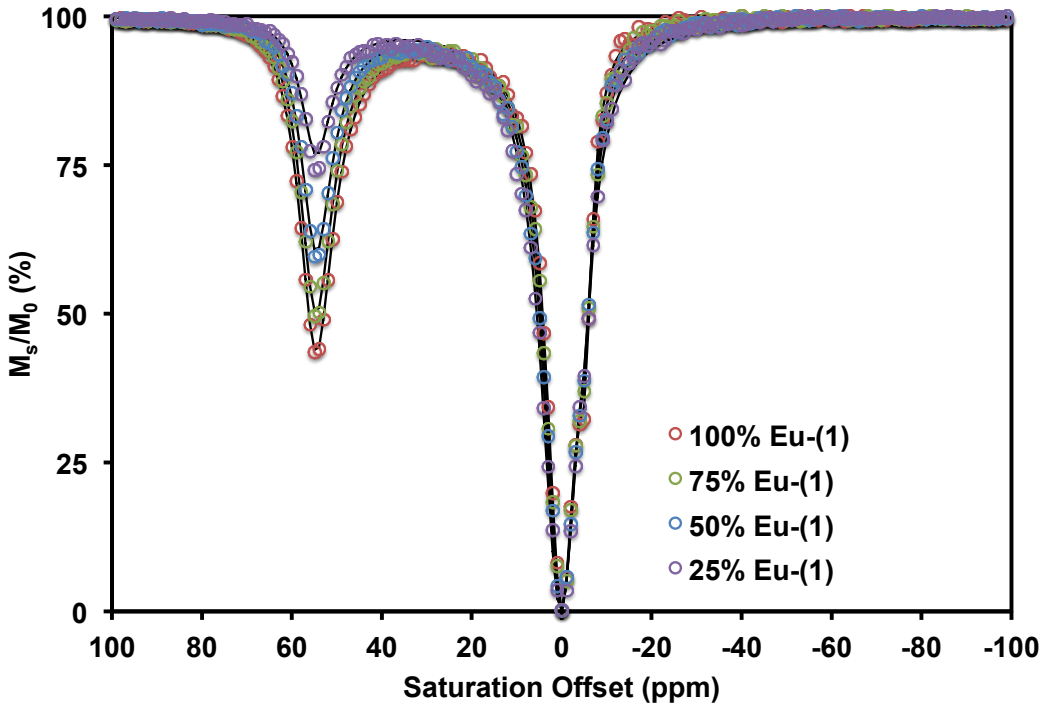

Figure S2. CEST spectra of aqueous solutions containing different proportions of Eu-(1) and Eu-(2) with $[Eu^{3+}]$ = 20 mM. Spectra were recorded at 25 °C, pH 7, B_1 = 14.1 μT, saturation time = 6 s.

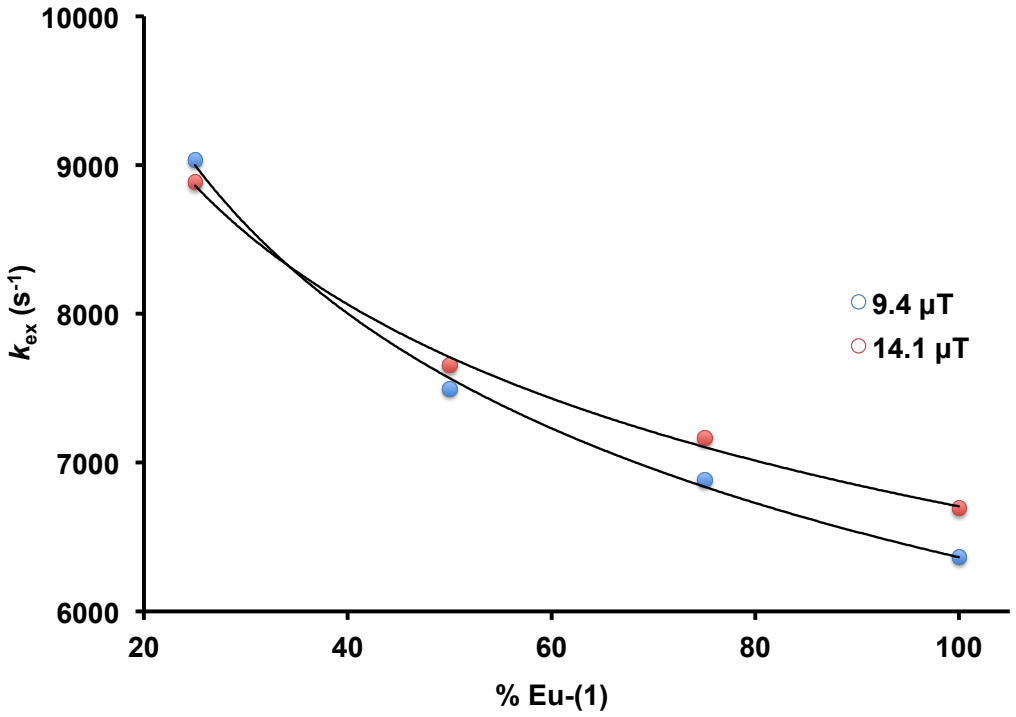

Figure S3. Calculated water exchange rates of SAP isomer of Eu-(1) obtained by fitting CEST spectra recorded at 9.4 and 14.1 μT to the Bloch equations (3-pool model; Bulk water, SAP isomer bound water, and amide protons.

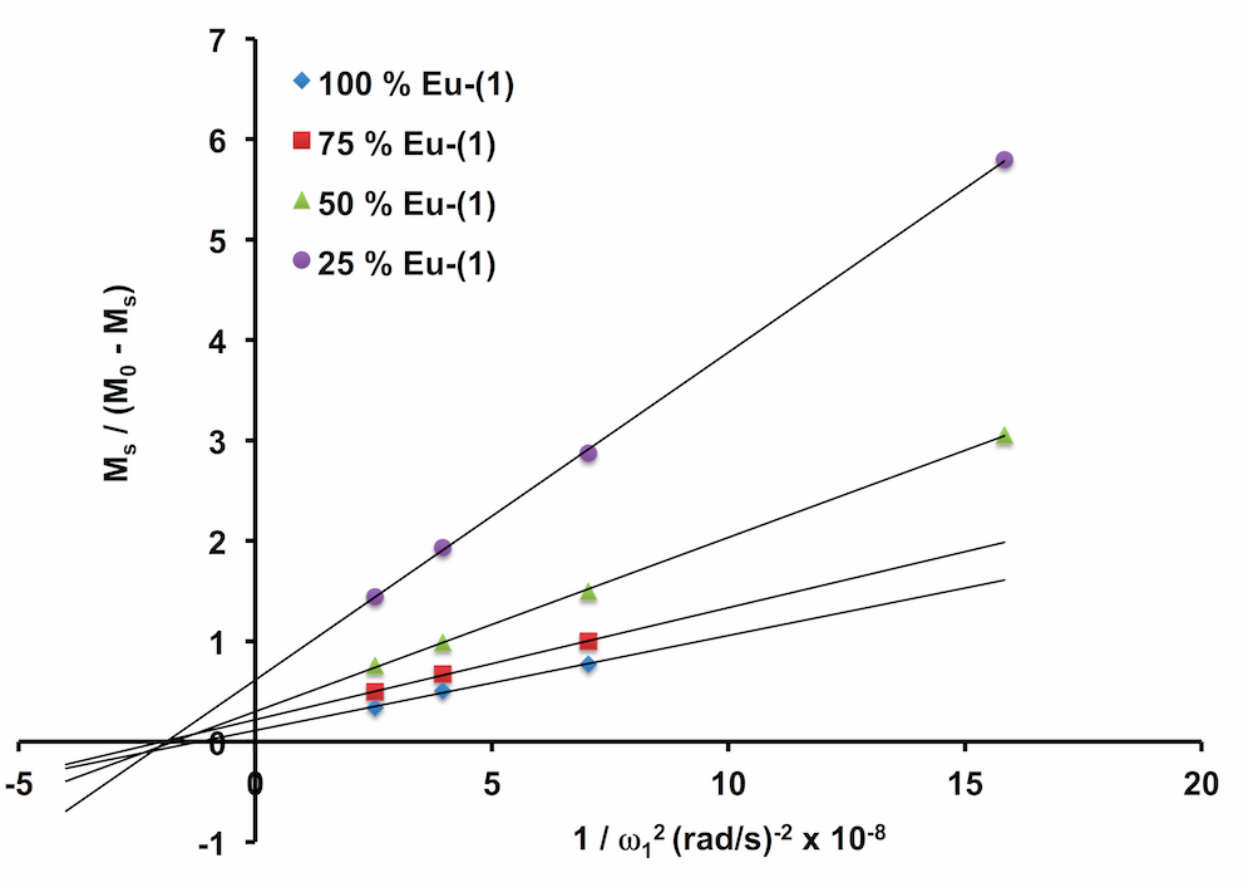

Figure S4. Omega plots for the same four samples containing varying proportions of Eu-(1) and Eu-(2). CEST spectra were collected using variable B_1 values ranging from 9.4 – 23.5 μT.

REFERENCES

(1) Aime, S.; Delli Castelli, D.; Terreno, E. *Angewandte Chemie-International Edition* **2002**, *41*, 4334-4336.

(2) Mani, T.; Tircso, G.; Togao, O.; Zhao, P.; Soesbe, T. C.; Takahashi, M.; Sherry, A. D. *Contrast Media & Molecular Imaging* **2009**, *4*, 183-191.

(3) Dixon, W. T.; Ren, J.; Lubag, A. J. M.; Ratnakar, J.; Vinogradov, E.; Hancu, I.; Lenkinski, R. E.; Sherry, A. D. *Magn. Reson. Med.* **2010**, *63*, 625-632.